THE SURVIVAL OF ARTEMIA POPULATIONS IN RADIOACTIVE SEA WATER

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With salt-water organisms there have been few attempts to check conclusions based on data from the more traditional species used in radiation genetics. Possibly this traces to difficulties in maintaining stocks of known ancestry, although we have met no serious maintenance problems with strains of *Artemia*. The brine shrimp thrives without running water and thus we avoid escape of zygotes or the loss of floating gametes. Furthermore, their ability to cope with environmental stresses, including ionic and osmotic changes (Lochhead, 1941), suggested that *Artemia* would be ideal for experiments in which radioisotopes would be added to sea water.

The persistence of mass cultures and the fitness components obtained from pair mating tests are reported below for *Artemia* whose ancestors survived sea water to which either P^{32} or Zn^{65} had been added. These isotopes were used because radiation ecologists have shown particular concern about their presence in the vicinity of nuclear reactors and atomic test sites (Gong, *et al.*, 1957; Davis, 1958; Davis *et al.*, 1958). For comparison and contrast, data from experimental populations whose ancestors had received acute exposures to x-rays are also included.

Considering fitness to be measurable as the number of mature offspring left by tested parents, we obtained a basis for comparison between descendants of control and irradiated *Artemia*. There is no evidence of increased fitness over controls for any experimental population in sea water, diluted sea water or brine.

Materials and Methods

Stock origin and maintenance

Our *Artemia salina* stocks originated from commercial dry cysts of the diploid amphigonic California strain. Although mass culture techniques were explored earlier (Grosch and Erdman, 1955), the oldest cultures extant date from 1957. One of these, number 3 used in the present study, has been maintained from its beginning in the same 5-gallon rectangular battery jar. Additional available control cultures were begun in 1959 in the same cylindrical gallon jars now containing them. Of the several available, number 8 has been used in the present study. Control maintenance has not been a problem. In fact, five control cultures have been discarded due to limitations in space. All control cultures were started from several

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hundred dry cysts, and as many as 300 well developed *Artemia* have been counted in a gallon control at the height of the summer. Earlier, in June, the first group to mature tends to be somewhat smaller, numbering 50 to 100.

Using ten pairs of adults per three liters of sea water seemed the most feasible approach to setting up radioisotope experiments. A series of doses can be instituted simultaneously without endangering persistence of the control culture from which the pairs of adults are removed. Culture #3 provided the parents for all experimental cultures to date. Table I summarizes these cultures and the nature of their treatments.

TABLE I

Inception and subsequent history of three-liter experimental cultures of Artemia. T = tested by pair matings. DNS = did not survive. ? = survival questionable

1958	1959	1960	1961	1962
μc. P^{32} added				
30 A	T	T	T	testing
B	T	T	too few to test	DNS
	30 μc. P^{32} added	DNS		
		30 μc. P^{32} added	DNS	
			30 μc. P^{32} added	
40				
60				
90	T	T	T	DNS
120	DNS			
	μc. P^{32} added			
	30			
	90	too few to test	T	testing
	120	became extinct		
	150	DNS		
	200	DNS		
	450	DNS		
	r, x-ray			
	1000	T	T	testing
	2500	DNS		
	3000	DNS		
		r, x-ray		
		1000		
		2500	DNS	
		3000	DNS	
		μc. Zn^{65} added		too few to test
		30	T	
		60	DNS	
		90	DNS	
		120	DNS	
			μc. Zn^{65} added	
			30	?
			60	DNS
			90	DNS
			120	DNS

In 1958, P^{32} in phosphate form was added to a series of three-liter (3-L.) cultures at the following levels: 30, 40, 60, 90 and 120 μc. The 30 μc./3-L. culture gave rise to two subcultures known as "A" and "B" which differ by one generation. In August, when F_3 larvae became evident, the F_2 parents were removed to another 3-L. jar where they produced cysts which overwintered. This culture was designated "A." The culture derived from the cysts produced by the F_3 remaining in the original jar has been known as "B."

In 1959 duplicate experiments were set up at 30, 90, and 120 μc./3-L. In addition higher doses were given to check on the suspected limits of tolerance: 150, 200, and 450 μc. In 1959 and each successive year descendants from the 1958 30-μc. dose were subcultured and given a repeated dose of 30 μc. of P^{32}

Zn^{65} in chloride form was added to four 3-L. cultures in 1960 at the following levels: 30, 60, 90, and 120 μc. This was repeated in 1961.

The x-ray exposures were made in 1959 and 1960. Each year, ten pairs of adults were given three doses each from the Woods Hole generator. It operated at 30 ma. and a 200 Kv. peak with an inherent filter equivalent to 0.2 mm. of Cu. Delivered in a few minutes, the acute doses were 1000 r, 2500 r, and 3000 r, respectively below, near and above the dose found sterilizing for adult females (Grosch and Erdman, 1955; Grosch and Sullivan, 1955). All cultures have remained indoors, shelved near, but not in, windows which receive sunlight for half of the day. The cultures were untouched from September until June. During this winter period, the water gradually evaporated until only an inch of saturated brine remained, along with crystalline salt deposits and colorless algal debris. Persistent adults were seen only occasionally. The cultures were reconstituted from the cysts deposited on the sides of the container upon filling with distilled water to the original high water line. The salts dissolved with stirring.

In general the procedure followed a natural sequence of events described by Boone and Baas-Becking (1931) for California salterns, where "winter eggs" are left along the high water marks, to "swell and burst" in the spring when freshets dissolve the salt crust and the environment reaches a favorable salinity. In laboratory cultures it has seemed necessary to remove any large masses of putrefying algae soon after emergence of the *Artemia* larvae. Earlier removal may not be advantageous because some cysts can be trapped in the mass.

Pair mating tests

In order to study reproductive capacity and adult life span, pairs of young adults were moved to quart jars from the large mass cultures upon reaching sexual maturity. Transfer was by dipping or pouring because adults are easily injured by pipetting. The pair matings were inspected daily until the death of both animals. Upon their appearance, broods of live young were counted and removed to separate containers, to determine their ability to complete development. If cysts appeared, they were filtered from the culture, dried, counted and resuspended in dilute sea water for hatchability determinations. When broods reached sexual maturity, the offspring were counted again and sexed.

Control data gave us reason to believe that quart jars are entirely adequate for survival records, but to make sure, crowding experiments were performed with much smaller 4-ounce jars. The experiments, repeated three times, involved a

series of 2, 4, 8, 16, 32, 64, 128, 256 nauplii per 4 ounces of sea water. Results of crowding were evident when groups involved more than 32, but this took the form of repressed growth and delayed maturity rather than death. A feedback phenomenon, such as reported by Rose (1960) for fish and amphibia, is suggested.

During the summer all cultures were fed daily with yeast suspension, roughly at the rate of one drop per adult, added to the culture water. In addition they ate the volunteer algae present in the cultures. In fact pair matings and their offspring were maintained under constant illumination from banks of fluorescent tubes as customary in algae culture. The temperature on warm days reached 28° C. under such circumstances, at night and on cool days it fell off a degree or two. The temperature for mass cultures elsewhere in the room varied more than this during the growing period, averaging 25° C. but rising to 30° for afternoons when sun reached the cultures and falling to 20° on cool nights. This is much like the range in temperatures experienced by Bowen's (1962) cultures.

Pair mating tests were performed in sea water at the convenient specific gravity of 1.02 until 1961 when the comparisons were also made at higher and lower specific gravities within the range of adaptation found by earlier investigators (Jensen, 1918; Bond, 1932). Sea water was diluted to a specific gravity of 1.01 with distilled water. For high salinities NaCl was stirred into sea water to raise the specific gravity to 1.07 and 1.12. Adults typically survived transfer directly to 1.01 (lower) or 1.07 (higher) specific gravities, but rarely survived transfer to the 1.12 brine. Therefore gradual conditioning was attempted by daily additions of twelve successive equal doses of salt until a specific gravity of 1.12 was reached. However, only about 10% of the young adults used survived such conditioning.

Results

Survival of cultures

Control cultures have been prolific and maintain themselves without difficulty. On the other hand, experimental cultures may be sparsely populated and those experiencing higher levels of radiation quickly trend to extinction. This results from reproductive failure rather than any other obvious influence on the treated adults. A summary of cultures begun and those which failed to survive is given by Table I.

In the P^{32} series, 3-L. cultures above 90 μc. have failed to survive. This limit was demonstrated for both the 1958 and 1959 series of experiments. The most persistent case was a sparse population in a 120-μc. jar which survived the 1959–60 overwintering but during the summer of 1960 did not expand successfully. Subcultures of 30-μc. experiments have not survived a repeat dose of 30 μc. of P^{32}. Furthermore, after 14–15 generations, the 1958 series of P^{32} cultures have entered a period of decline and seem to be on the verge of extinction. Cultures of the 1959 series which have gone through only nine generations appear to be in better condition.

In the Zn^{65} series *Artemia* cultures have survived only the lowest dose, 30 μc./3L. In the x-ray series persistent cultures have not been obtained from *Artemia* receiving more than 1000 r. In one culture from *Artemia* which had received 1000 r, nine generations have now elapsed. In 1960 the culture whose ancestors received 1000 r of x-ray appeared superior to the culture begun at the same time

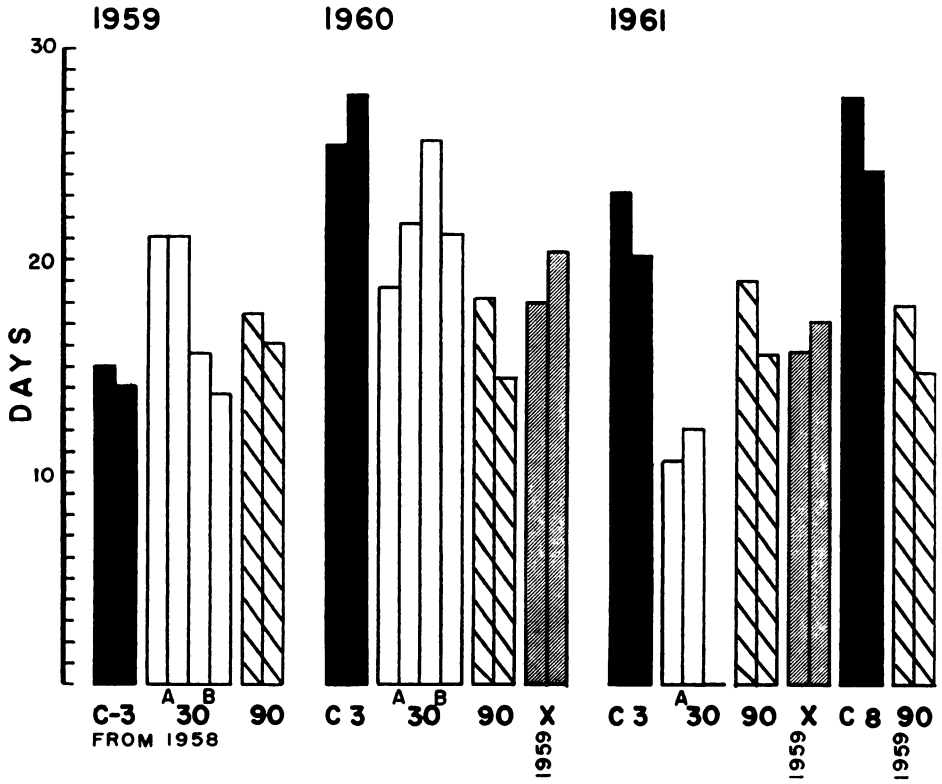

FIGURE 1. Survival of adult *Artemia* after transfer to quart jars for pair mating tests. The summer in which the data were obtained is identified by the year shown at the top; the cultures from which the animals were taken are designated along the bottom of the figure. C indicates control; X stands for x-rayed; 30 and 90 refer to the μc. of P^{32} added to respective 3-liter cultures at the start of the experiments. Female survival is given by the right bar of each pair.

incorporating 90 μc. of P^{32}. The x-ray culture provided adequate numbers of pairs for testing during a period when the P^{32} jar was too sparsely populated.

Duration of life

The average number of days between their transfer to quart jars and the death of members of mated pairs of *Artemia* is taken as a measure of adult life span (Fig. 1). Typical standard errors associated with these values range from 2.40 to 2.49 days for males and from 3.12 to 3.65 days for females. In 1959, experimental animals lived as long as or longer than the controls from culture #3. In subsequent years, individuals whose ancestors were subjected to radiation tended to die sooner than #3 controls. In 1961 an additional control, #8, was sampled. This has been maintained in exactly the same size and shape of jar as all experimental cultures. As shown, the adults withdrawn from #8 lived even longer than those from #3. Therefore, size and shape of container are ruled out as influences in poor life-span of experimental adults.

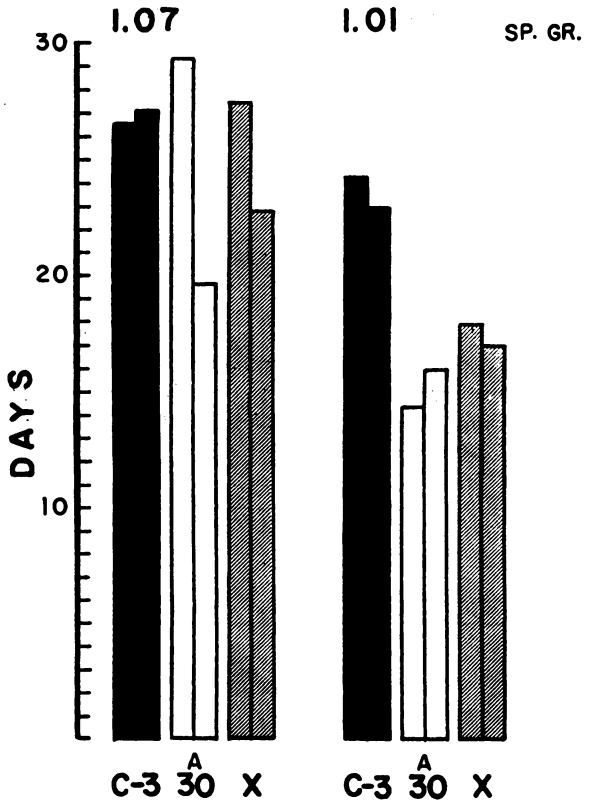

FIGURE 2. Survival of adult *Artemia* used for pair mating tests in sea water to which NaCl had been added (1.07 sp. gr.) and in diluted sea-water (1.01 sp. gr.). The respective culture supplying the animals is designated along the bottom: C-3 indicates control #3, A 30 refers to 30 μc. added to a 3-L. culture in 1958, X stands for the culture whose original parents were x-rayed in 1959. Female survival is given by the right bar of each pair.

Pair matings from the 1958 cultures receiving 90 μc. of P^{32} have given relatively consistent life span records in successive years. This is true also for the duplicate experiment begun in 1959. Furthermore, similar life spans have been obtained from the acute dose of 1000 r of x-rays. On the other hand, the cultures derived from a 30-μc. P^{32} treatment have varied over the years of study. Recently adult life span has proved brief in ordinary sea water. Additional results which are not included on the figure are for the 30-μc. Zn^{65} culture begun in 1960. Average survival of males was 18.6 ± 3.95, and 19.3 ± 2.28 days for females.

When brine of 1.07 specific gravity was used for tested pairs, adult life span was prolonged. The proper comparison is between the 1.07 results of Figure 2 and the 1961 results of Figure 1 for the same three cultures. The standard errors for Figure 2 values are within the range stated above for Figure 1 values.

In brine of higher specific gravity, 1.12, the two pairs of control adults which survived conditioning had lengthy spans of life: 31 and 36 days for males, and 28 and 24 days for females. P^{32} animals did not do as well. Four conditioned pairs

averaged 22.5 and 13.2 days for males and females, respectively. From the x-ray population, the one pair conditioned died after only 11 (female) and 15 (male) days.

Figure 2 also presents survival of adults in dilute sea water of 1.01 specific gravity. These values are better but do not impressively exceed the 1961 sea-water results for the same culture (Fig. 1). It is impossible to run all tests simultaneously so some improvement might be due to increased experience of the assistant. However, in this case less variability might be expected. Instead, standard errors exceed 4 days, and for males of control #3 is a high 5.22 days.

Components of fitness

Life span is part of the story, but it is possible to examine the various aspects of reproductive failure more directly. The summaries in Tables II and III indicate whether mated pairs are likely to give rise to sexually mature offspring. Adaptive values epitomize the reproductive efficiency of a genotype in a certain environment.

TABLE II

Reproductive behavior of Artemia cultures as revealed by pair mating studies in sea water

Cultures treated at the date indicated	Number of broods per pair	Zygotes voided *		% Survived to adult	Mature adults per pair	Adaptive value
		Per pair	Per brood			
Results of pair matings in 1959						
Control #3	1.2	176.9	81.7	24.3	43.0	1.00
30 μc./3L. 1958 "A"	0.9	31.6	27.4	24.4	7.7	0.18
"B"	0.7	85.1	52.7	26.0	22.1	0.51
90 μc./3L. 1958	0.6	31.8	39.8	20.0	6.4	0.15
Results of pair matings in 1960						
Control #3	2.6	387.4	149.0	43.1	156.97	1.00
"A"	1.6	165.9	103.7	27.6	45.79	0.29
"B"	2.6	381.9	146.9	31.5	120.30	0.77
90 μc. 1958	1.3	110.1	84.7	30.3	33.36	0.21
X- ray 1000 r. 1959	1.0	106.5	106.5	31.2	33.32	0.21
Results of pair matings in 1961						
Control #3	2.2	179.5	81.6	50.3	90.3	1.00
Control #8	2.4	164.6	68.6	76.5	125.9	1.39
"A"	0.3	12.8	42.7	57.6	8.7	0.10
90 μc. 1958	0.2	6.1	30.5	0	0	0
X-ray 1959	0.5	19.93	39.9	14.5	2.9	0.03
90 μc./3L. 1959 Zn^{65}	0.3	7.08	28.3	32.7	2.3	0.03
30 μc./3L. 1960	1.17	59.2	50.6	72.6	42.9	0.58

* Zygotes voided is used to refer to the number of nauplii and cysts deposited.

TABLE III

Reproductive behavior of Artemia cultures revealed in 1961 by pair mating studies in salt waters of higher and lower specific gravities than sea water

Cultures tested	Number of broods per pair	Zygotes voided *		% Survived to adult	Mature adults per pair	Adaptive value
		Per pair	Per brood			
Specific gravity 1.01						
Control #3	2.2	228.6	103.9	47.4	108.3	1.00
"A" 1958	0.8	20.5	24.6	26.4	5.4	0.05
X-ray 1959	1.1	51.2	46.6	62.8	32.2	0.30
Specific gravity 1.07						
Control #3	2.8	182.5	65.3	51.7	94.3	1.00
"A" 1958	1.9	107.3	60.4	61.9	66.4	0.70
X-ray 1959	2.1	85.4	41.0	69.0	58.9	0.62

* Number of nauplii and cysts deposited.

As defined (Dobzhansky, 1951) adaptive value is the relative capacity of carriers of a given genotype to transmit their genes to the gene pool of the following generations. On this basis we have taken our evidence of the average number of mature progeny produced per pair, assigned the unit value to control #3 and made the pertinent comparisons within each year.

Aspects in which a cultured population is deficient are revealed in these experiments where all voided zygotes and all adults developing therefrom are counted. Table II presents results for pair mating tests in three successive years, using sea water of 1.02 specific gravity. Table III presents data from 1961 experiments at lower and higher specific gravities.

Experimental populations have never approached the controls in their production of live offspring. "B" came closest in 1960 when as many broods were deposited and the number of zygotes per brood was only slightly lower. However, the curbing influence was revealed in fewer offspring surviving to adulthood. Subsequently decline of this culture has been so drastic that in 1961 it did not produce enough adults to allow pair mating tests. Adequate numbers of adults for the 1961 pair mating tests were provided by the survivor of the highest P^{32} level of 1958, 90 μc./3L., but Table II demonstrates poor performance in all the aspects considered. The culture which gave an adaptive value of zero in 1961 tests did not survive overwintering to 1962.

A small number of broods, a form of infecundity, has appeared for various experimental cultures tested by pair matings. At the same time, the decrease in the number of zygotes per brood may not be severe. Indeed, in 1961 "A" was producing considerably larger broods than it was in 1958 and survival to adulthood was better than for #3 control, yet relatively few adult offspring were obtained, chiefly because parents which were fertile produced only single broods. Earlier indications of the importance of fecundity came in 1959 when experimentals and controls

differed only slightly in larval lethality, and in 1960 when there was little difference in larval lethality among various experimental groups. Finally in 1961, larval survival for "A" and for Zn^{65} pair mating tests surpassed that for the #3 control although neither adaptive value at all approaches the control value.

Table III demonstrates that *Artemia* react differently in their reproductive abilities when the specific gravity of the medium was made higher or lower than the convenient 1.02 of sea water. Particularly notable were the improvements in all reproductive aspects for "A" and the x-ray parents when brine of 1.07 specific gravity was used. Improvements, except in number of zygotes per brood, were also seen for control #3.

On the other hand, brine of 1.12 specific gravity did not improve the reproductive ability of *Artemia*. One or more broods were produced for the few pairs conditioned to this salty brine, but survival to adulthood was poor: 26.1% for controls, 9.3% for "A" and zero for larvae from x-ray parents. The number of offspring per brood was not good: 43, 26, and 14.5, respectively.

Hatchability of cysts

Although cysts occur regularly in the mass cultures which evaporate slowly during the winter, under the conditions of the pair mating tests, "winter eggs" appeared only occasionally. When obtained they formed the last brood or a part of it and contributed only a small fraction of the total zygotes voided. Table IV presents emergence or hatchability of the cysts, along with the number of mated pairs producing cysts. On the basis of the 1960 records it might seem that when females have a longer life span (Fig. 1) they are more likely to deposit an encysted brood, although this is not borne out by our subsequent experience. For example Control #8 in 1961 averaged 24+ days for female survival but deposited no cysts. Furthermore in brine of 1.07 specific gravity only 2 of 10 #3 control females deposited cysts, although the average survival for both sexes was 26–27 days.

If there is no sperm storage (Bowen, 1962) male life span could be a limiting factor on cyst deposition. However, from this standpoint the 1960 #3 control would drop back to 25 days for effective pair survival, which is not significantly different from the #8 1961 control and shorter than the 1961 #3 control values in brine (1.07 specific gravity).

TABLE IV

Cyst deposit and emergence from cysts

Source	1959		1960		1961	
	% emerged	No. laying cysts per 15 mated	% emerged	No. laying cysts per 20 mated	% emerged	No. laying cysts per 10 mated
Control #3	41.9	6	46.4	17	46.0	6
30 μc. P^{32} A	15.4	1	29.2	12		0
30 μc. P^{32} B	24.9	3	47.5	16		
90 μc. P^{32} 1958	18.1	1	58.5	6		0
X-ray			49.5	8	28.4	2
Zn^{65}					45.5	1

In spite of the inconsistent deposit of cysts by females maintained in isolated jars under continuous illumination, some insight is provided concerning survival of mass cultures. In 1960, the year when a large proportion of pairs produced cysts, tests of three out of four experimental cultures showed hatchability above the control values. On this basis hatchability does not seem to be a major influence upon survival of a culture.

Control values between 40 and 50% hatchable cysts are not unexpected in unselected samples. Unselected commercial samples of cysts from natural populations may give even lower hatchability. Flotation or some other method of eliminating deficient or empty cysts seems necessary to improve hatchability.

Sex ratio

A subtle difference between populous cultures and those which appear headed for extinction is revealed by summarizing the sex ratios of offspring reaching maturity. A vulnerability of females in treated populations suggests the segregation of deleterious induced recessives in the heterogametic sex (although the author is aware that the question of female heterogamety in *Artemia* is still controversial).

Table V demonstrates that the sex ratio tends to favor males when the parental pairs tested are drawn from cultures whose ancestors were irradiated. In eight out of ten sets of pair mating tests the control value for the particular year is exceeded. Chi square determinations provide significant values for 1960 "A" and

TABLE V

Sex ratios given as the ratio of males to females, and chi square values calculated from the original data

Origin of parents	1959	χ^2	1960	χ^2	1961	χ^2
Control #3	.82		.74		.91	
P^{32} 30 μc. A	1.25	2.069	.92	4.650*	1.31	3.450
P^{32} 30 μc. B	1.00	1.031	1.15	16.987**		
P^{32} 30 μc. 1958	.72	.124	.83	.907		
X-ray 1959			.68	.176	1.22	.860
Zn^{65} 30 μc.					1.02	1.380
Control #8					.95	.437
1.07 sp. gr. brine						
Control #3					.91	
A 1958					1.34	9.903**
X-ray 1959					.93	.006
1.01 sp. gr. dilute sea water						
Control #3					.88	
A 1958					1.40	3.262
X-ray					.87	.0004

* = significant.
** = highly significant.

"B" results as an indication that deviations are more than subtle in those cases. Note that in controls, more adult females were produced than males, while experimental cultures favor males.

At both higher and lower specific gravities the sex ratio favors males in tests of Culture A originally derived from ancestors exposed to 30 μc. of P^{32} in 1958. The chi square value for the 1.07 specific gravity test is highly significant. The culture from x-rayed ancestors, which at present is more prolific than "A", shows sex ratios not significantly different from those of #3 controls run at the same specific gravities.

Discussion

The fact that *Artemia* cultures derived from radioisotope- and x-ray-exposed ancestors are doing poorly may be viewed from several aspects although the problems of waste disposal, ecological disturbance and population genetics are interrelated.

In practice, where isotopic concentrations have been determined in the environs of the Hanford, Washington, nuclear plant, concentrations in the effluent water are much lower than the levels used for our experiments. Bustad (1960) reports 2×10^{-8} μc./cc. for P^{32} and 1×10^{-7} μc./cc. for Zn^{65}. These are activation products rather than discharged wastes. Another example is White Oak Lake which received effluents from Oak Ridge, Tennessee. Wastes here include fission products and transuranic elements, yet the average concentration in the water was estimated at 10^{-3} μc./cc., lower by at least a factor of ten than any of our experiments.

On the other hand, a document considered when the experiments were planned (NAS-NRC, 1959) gave a maximum permissible concentration of Zn^{65} in drinking water, 6×10^{-2} μc./cc. or 180 μc./3L., a level twice that at which *Artemia* can persist, and six times that which makes population survival difficult. The generalized concentration factor employed for invertebrates provided a more acceptable value of 1.2×10^{-4} μc./cc. as the permissible sea water concentration for Zn^{65}. In contrast, even without the invertebrate concentration factor, the MPC of P^{32} in drinking water (2×10^{-4} μc./cc.) was placed well below any level yet studied with *Artemia* populations. The recommendations were based on Handbook 52 of the National Bureau of Standards, now superseded by Handbook 69 in which permissible levels have been reduced for many isotopes.

In waters studied by ecologists it was the highest trophic levels which were damaged. Although species of fish were disappearing from White Oak Lake, and shortened life span and poor growth were reported for others, populations of aquatic insects were able to survive in spite of impressive concentration factors (Buchsbaum, 1958). Enormous doses of radiation may be necessary to destroy completely a primary trophic level such as an algal-protozoan community. No significant physiological or morphological damage to marine algae was demonstrated after the Bikini atomic tests (Blinks, 1952), although damage to the hereditary mechanism was not assessed. Doses such as those employed in the present experiment apparently seem in the range necessary to interfere with the primary consumers of the second trophic level, *Artemia* for example. Furthermore, the approach of the population geneticist is needed to reveal the nature and extent of the damage. Experimental *Artemia* showing no visible evidence in numbers or appearance of individuals for one or several generations, may carry hidden genetic damage responsible for subsequent decline to a dangerously small population.

Diptera have been the preferred material for such research, even for estimating genetic damage from the Caroline Islands atomic tests (Stone *et al.*, 1957; Stone and Wilson, 1958). Experimental procedures included population sampling by brother-sister matings. Reproductive performance, studied under laboratory conditions, revealed that direct irradiation and fallout damaged *Drosophila ananassae* populations severely. Many mutants and gene combinations interfered with development to adulthood, a difficulty demonstrated again in the present *Artemia* experiments. In spite of viability problems, the *Drosophila* populations have managed to return to normal reproductive performance, presumably through the operation of natural selection. The flies required from 26 to 161 generations to achieve reproductive recovery. Little more than half the lower number of generations has elapsed for the oldest *Artemia* culture. It will be interesting to see whether any of the irradiated *Artemia* populations can accomplish a recovery to normal levels of reproductive performance.

For *D. ananassae* no consistent relation of egg counts to genotype was detected (Stone *et al.*, 1957) although survival-extinction predictions for *D. melanogaster* are based in part upon fecundity (Wallace and Dobzhansky, 1959). Since the maximum number of possible offspring depends upon the number of functional eggs produced, there is a certain number of eggs required per female if the population is not to become extinct when exposed to a given amount of radiation. Fecundity as well as zygote viability is under genetic control and subject to irradiation damage, so that two dose-dependent aspects of survival are interrelated. With the exception of "B" in 1960, our experimental *Artemia* cultures have shown poor fecundity from the beginning. Possibly in a viviparous animal this matter is more serious than in an oviparous form. Insurmountable crises in development may occur which result in elimination of the zygote before deposit. Indeed, our category of "zygotes voided" may really reflect early embryo death and resorption as well as egg productivity. The cysts, which are often incorrectly called "eggs," are really embryos as far along as the blastula stage.

The price paid for the elimination of detrimental and lethal factors from a population is death of individuals, actual or potential. Our *Artemia* populations may now be paying this price. Controversy exists concerning (a) the retention of seemingly deleterious chromosomes for virtue of their characteristics in heterozygous individuals (Wallace, 1956), and (b) whether ambivalent mutants exist which impair fitness when homozygous but improve that of their heterozygous carriers (Wallace and Dobzhansky, 1959). High adaptive values for irradiated *Drosophila* populations have been reported (Wallace and King, 1951; Wallace, 1951), and the adaptive value for one acutely irradiated population even exceeded control values. In this case an x-ray dose of 1000 r was delivered to females and seven times that dose to males. In contrast to the *Drosophila* results, experimental cultures of *Artemia* whose ancestors received 1000 r. to both sexes are clearly inferior to control populations. Indeed, for experimental *Artemia,* none of the adaptive values approach the high values reported for *Drosophila.* However, here again a comparable number of generations has not elapsed. By 1956, Wallace's populations had been followed for 150 generations; by 1959, 200 generations had elapsed. In addition there are a number of other features, such as size of organism and irradiation in water vs. air, which complicate a comparison. Furthermore

Wallace's *Drosophila* populations involved a contrived genetic background, an intentional isogenicity not readily obtainable with other organisms. Also, from a cytological standpoint it may be significant that *Drosophila* has a small number of chromosomes, some of which are long, possibly an ideal situation for fixation of chromosomal polymorphism. In contrast, *Artemia* has a large number of short chromosomes.

Selection experiments clearly indicate the accumulation of genetic lethals in irradiated laboratory stocks of *Drosophila* (Muller, 1950), but Wallace argued that fitness of a population consisting mainly of heterozygous individuals may be excellent, provided the population is large enough so that segregation of detrimental homozygotes will not threaten its existence. Perhaps our populations of several hundred *Artemia* are dangerously small, but this reflects our decision to devote facilities and efforts to a number of cultures encompassing a range of treatments, rather than to a few enormous populations which might have been given treatments too low for sharply defined comparisons. Actually, results from populations of limited size may be especially pertinent for practical considerations in other organisms. Although seasonally dense populations of *Artemia* occur in some salterns, such cases may be exceptional in present day ecology. Field studies have shown that most of the species present in a locality are represented by only a few individuals (Williams, 1953).

Doubt has been cast on improvement resulting from irradiation through a neoclassical version of heterosis. If mutations increasing the viability of the heterozygote are not demonstrable under favorable conditions for their detection, they are not too helpful an explanation of conditions in natural and experimental populations (Muller and Falk, 1961). Only decreases in the average viability of an otherwise homozygous *Drosophila melanogaster* genotype were obtained for radiation-induced mutations in heterozygous and unselected conditions (Falk, 1961). Furthermore, in laboratory conditions no significant influences on heterozygote viability were demonstrable for *D. willistoni* lethals, whether natural or induced (da Cunha *et al.*, 1959). In plants, Stadler's (1932) pessimism about the damaging aspects of radiation-induced mutation is traditional, although for cultivated crops desirable traits may emerge from irradiated populations under the practice of artificial selection (Gustafsson, 1947; Sparrow and Singleton, 1953; Konzak, 1954; Gregory, 1956). Finally, to date, only detriment has been demonstrated for *Artemia* cultures descended from irradiated ancestors.

A notable point concerning *Artemia* biology has emerged from these studies. Increased life span and improved reproductive performance in brine (1.07 specific gravity) indicate favorable aspects in addition to a lack of predators (Lochhead, 1941) in the niche with which *Artemia* is associated. Other recent investigators feel it desirable to culture *Artemia* in water saltier than sea water. Bowen's (1962) standard procedure is to add NaCl as we have done. After trials with different concentrations, Goldschmidt (1952) adopted a standard specific gravity of 1.04 obtained by evaporation.

Summary

1. Results are presented for four years of study on the survival of *Artemia* cultures when ancestors have been exposed to a series of doses of either radioisotopes

or x-rays. Cultures were begun by transferring 10 pairs of adults from a control culture to a 3-liter jar of sea water. Ordinarily, within a generation this gives rise to a culture of several hundred animals.

2. Three-liter cultures did not persist if more than 90 μc. of P^{32} or more than 30 μc. of Zn^{65} have been added. Subcultures of 30 μc. of P^{32} per three liters did not survive a second dose of 30 μc./3L. Also, cultures failed if 2000 r or more of x-rays were delivered to the 10 pairs of adults used to institute the culture.

3. The treatments investigated had no obvious effect upon the original adults. Decline and extinction of the cultures occurred at the first or subsequent generations of offspring.

4. In order to assess reproductive failure, pairs when sexually mature were transferred from the 3-L. cultures to quart jars. All zygotes voided were counted and hatchability was determined for any cysts deposited. Each brood was transferred to a separate container. Progeny surviving to adulthood were counted again and sexed.

5. (a) Decrease both in number of zygotes voided and in survival to adulthood contributed to low adaptive values for experimental organisms. .

(b) The sex ratio among offspring tends to favor females in control and males in experimental material.

6. Routinely the convenient specific gravity of 1.02 has been used for pair matings and spring reactivation of mass cultures. In 1961 pair mating tests were run in dilute sea water of 1.01 specific gravity and in sea water to which NaCl had been added to reach a specific gravity of 1.07. Both life span and reproductive behavior were improved in brine of 1.07 specific gravity. However, attempts to condition adults to saltier brine of 1.12 specific gravity were rarely successful and reproductive performance of the few shrimp conditioned was poor. Evidently there is an optimum brine range for *Artemia*, involving more fundamental biological aspects than previously reported.

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